Evolution oil dyskinetoplastic trypanosomes: how, and how often?

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Evolution of dyskinetoplastic trypanosomes: how, and how often?

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Trypanosoma equiperdum and *T. evansi* are close relatives of *T. brucei brucei* that have lost all, or critical parts, of their mitochondrial DNA (mtDNA, kinetoplast or kDNA), thus known as dyskinetoplastic (Box 1) [1].

Box 1

Dyskinetoplastic trypanosomes: mechanism of transmission, geographical distribution, and kDNA structure

Transmission of *T. equiperdum*and *T. evansi* between mammals occurs mechanically: venereally in case of the former and mostly via biting flies in case of the latter [4]. This permits their wide geographical distribution, while *T. brucei*, dependent on cyclical development in the tsetse vector, is restricted to sub-Saharan Africa. *T. brucei* kDNA consists of 40–50 maxicircles, the equivalent of mtDNA in other organisms, and thousands of heterogeneous minicircles, which encode the guide RNAs (gRNAs) required for editing of maxicircle-encoded mRNAs [10]. Different minicircle classes encode different gRNA sets, and *T. brucei* kDNA contains an estimated 300–400 classes [1]. All *T. equiperdum* and *T. evansi* strains show some degree of kDNA loss, ranging from intact networks with complete maxicircles, but minicircle homogenization (see table), to complete kDNA loss [1,3]. Originally, the terms dyskinetoplastic and akinetoplastic described cells completely lacking a kDNA structure. However, the minicircle homogenization in even the mild forms of kDNA loss in some *T. equiperdum* strains is expected to result in complete loss of fully edited mRNAs, except cox2 and MURF2 [3]. Therefore, most recent publications, including this one, refer to all *T. equiperdum* and *T. evansi* strains as dyskinetoplastic while reserving the term akinetoplastic for strains completely lacking detectable kDNA [1,3,12]. Mitochondrial gene expression is essential in *T. brucei* [1], and the compensation for its loss in dyskinetoplastic forms was suggested to involve mutations in the nuclearly encoded γ subunit of the mitochondrial ATPase complex (see table) [3,13], a hypothesis that awaits experimental confirmation.

In a stimulating article [2], Lun *et al.* suggested that dyskinetoplastic trypanosomes frequently and continuously evolve from *T. b. brucei* and proposed two alternative evolutionary scenarios. However, there is currently no conclusive evidence for a frequent *de novo* evolution of

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sustainable populations of dyskinetoplastic trypanosomes. Additionally, the proposed scenarios do not fully explain the link between mechanical transmission and dyskinetoplasty.

Two arguments were presented for a frequent emergence of *T. equiperdum* and *T. evansi*. Firstly, a phylogenetic tree based on SL RNA repeats showed *T. b. brucei, T. equiperdum* and *T. evansi* sequences intermingled [3]; however, other methods generally resulted in distinct clusters for *T. equiperdum*/*T. evansi versus T. brucei* [4–6]. Where present, *T. equiperdum* and *T. evansi* minicircle populations are largely homogenous [6], consistent with computer models predicting homogenization in the absence of selective pressure to maintain complexity [7]. Interestingly, three distinct ATP synthase γ polymorphisms were reported for naturally occurring dyskinetoplastic forms [3]. In five strains where both characteristics are known, predominant minicircle class and polymorphism are correlated (Box 1). If the functional significance of these polymorphisms and the generality of this correlation can be confirmed, this would be consistent with emergence of stable dyskinetoplastic forms on three independent occasions.

Secondly, Lun *et al.* reason that, without continuous emergence of new dyskinetoplastic forms, all extant strains should have reached the terminal akinetoplastic state, which is not the case. However, this rationale applies even if dyskinetoplastic forms have indeed been emerging continuously. The proposed elimination of akinetoplastic forms due to a selective disadvantage [3] is presently without biological basis. Thus, the considerable range of kDNA loss in present-day dyskinetoplastic strains is not inconsistent with (in evolutionary terms) relatively recent descendancy from a limited number of ancestors, possibly as a consequence of temporary introduction of camels or horses into tsetse areas [4,8].

Lun *et al.* suggested a plausible scenario for how *T. b. brucei* might become locked in the bloodstream stage, which required far fewer edited mRNAs for survival than insect forms [2]. In the absence of genetic exchange in the tsetse vector (which involves kDNA [9]), and as a consequence of random distribution and asymmetrical division of replicated kDNA [10], genetic drift will result in minicircle loss and, ultimately, loss of gRNAs required for differentiation into, and survival of, insect forms [7]. Evolution of RNA editing was rationalized as a protective mechanism against such loss of stage-specific genetic information [11], but that protection, if it exists, may not be complete. It therefore seems plausible that, as also suggested by Jensen and colleagues [12], after relocation of an infected animal to a tsetse-free area, and in the presence of conditions suitable for mechanical transmission, irreversible loss of certain minicircles will occur.

Nonetheless, important questions are not addressed by this model. At least the edited mitochondrial ATPase subunit 6 (A6) mRNA is required in bloodstream *T. brucei* [13]. It is obvious why dyskinetoplastic forms require mutations compensating for loss of A6, but what is the selective advantage that always favors these mutations, and kDNA loss, over faithful expression of this protein? Why do we not find mechanically transmitted *T. b. brucei* outside of Africa, with mitochondrial gene expression intact? Akinetoplastic cells have a possible ’replication advantage‘ since kDNA replication requires massive amounts of ATP, but this is not true for the intermediate forms with seemingly intact, albeit homogenous, kDNA networks. Furthermore, dyskinetoplastic forms still produce most, if not all proteins involved in kDNA replication and expression [3]. Is genetic drift alone sufficient to explain dyskinetoplasty? Or does kDNA loss perhaps somehow increase the efficiency of mechanical transmission?

A key factor favoring mechanical transmission by insects is high parasitemia in the host blood, which is directly proportional to the number of parasites sticking to the vector’s mouth parts and the likelihood of transmission [14]. *T. b. brucei* controls its parasitemia by differentiating into non-proliferative forms, which prolongs host survival and prepares the parasite for

infection of the tsetse midgut [15]. Differentiation involves mitochondrial activation and is impaired in dyskinetoplastic forms. Interestingly, mutations in the ATPase γ subunit that permit survival of petite-negative yeast in the absence of mtDNA result in rapid mtDNA loss in petite-positive yeast [16]. Possibly, occurrence of such a mutation in a bloodstream trypanosome that has found itself in a camel outside the tsetse area might result in loss of both kDNA and the capacity to differentiate and, therefore, higher parasitemia and an increased efficiency of mechanical transmission. Thus, the 'compensatory changes' would precede kDNA loss. Even in such a scenario, other factors would be important for a successful spread 'out of Africa'. Although both *T. b. brucei* and *T. evansi* are pathogenic to camels, infection with the latter often runs a more prolonged chronic course, ideal for efficient mechanical transmission [8]. Such host adaptations were certainly critical. More recently, treatment of infected animals with drugs known to bind kDNA (*e.g.* diminazene) may have selected for viable dyskinetoplastic forms, but appearance of most strains of *T. equiperdum* and *T. evansi* seems to predate use of these drugs [8]. Another mystery concerns the retention of maxicircles only in those dyskinetoplastic parasites found in tissue from horses and therefore classified as *T. equiperdum*. Investigation of the kDNA from different *T. vivax* isolates, a parasite transmitted both cyclically and mechanically within the tsetse belt, but exclusively mechanically elsewhere [14], might be illuminating.

In conclusion, although progress has been made in understanding the mitochondrial biology of *T. equiperdum* and *T. evansi*, the apparent links between efficient mechanical transmission, kDNA loss, and host and tissue specificity that have led to the enormous evolutionary success of these parasites remain unclear.

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Table

Species/strain	Origin	Minicircle class/type	ATP synthase γ polymorphism	Refs.
T. equiperdum STIB 818	China	A[a]	A281 → deletion	[3]
T. equiperdum ATCC 30019	France	A	A281 → deletion	[3]
T. evansi Antat 3/3	S. America	A	A281 → deletion	unpublished
T. equiperdum STIB842	unknown	B[b]	A273 → P	[3]
T. evansi KETRI 2479	Kenya	B	M282 → L	[3,6]

[a] Note that class A contains both *T. equiperdum* and *T. evansi*

[b] Lai *et al*. designated the predominant minicircle in STIB 842 as class B, but it is not related to the type B described previously for *T. evansi* KETRI 2479.